Johnstone (A. W.)

EXPERIMENTAL AND MICROSCOPICAL STUDIES ON THE ORIGIN OF THE BLOOD GLOBULES

BY

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EXPERIMENTAL AND MICROSCOPICAL STUDIES ON THE ORIGIN OF THE BLOOD GLOBULES.

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THE objects of this paper are to give the result of a repetition of Onimus' experiments on the "origin of the white blood corpuscles," and to place on record an account of an undescribed method of development that is constantly going on in the adenoid tissues. As given by Flint, these experiments of Onimus are as follows:

The serum from quickly-drawn blisters, after having been freed by filtration, etc., etc., from all its organized elements, is placed in bags of gold-beater's skin. These sacks are then placed in the subcutaneous tissues of rabbits, and after a sojourn of two or three days their serum is found to contain a variable number of leucocytes.

His conclusions are that the corpuscles have sprung up *de novo* from the blastema, and by analogy he argues that there is a spontaneous generation going on in serum where-ever it is found.

I have repeated these investigations, and in two directions have pushed them further than their author; that is, instead of the blastema, in the course of the experiments I used four different liquids, and in all cases, besides the fluids, I examined the gold-beater's-skin after its removal.

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In addition to the serum I used a weak solution of chloride of sodium in water, a mixture of this with the white of an egg, and lastly the clear part of the egg alone. The animals used were cats; the length of experiments from 17 to 50 hours; the thickness of the enclosing membranes was in most instances one, but in two cases two, layers of the gold-beater's skin. In all cases I examined both membrane and blastema before the introduction to the cat, and thus made sure that no organisms were present. My results were that in every case, except where I used a varnished membrane, I found leucocytes in the blastema, and wherever they were found in the liquid the walls of the enclosing bag were sure to be crowded with the same organisms.

The only things that seemed to influence the number of the corpuscles were the condition of the containing membrane and the length of time the sack remained under the skin. If these conditions were the same there were just as many corpuscles in the solution of chloride of sodium, or the egg mixtures, as there were in the serum. In the cases where the skin was doubled after a longer time than was ordinarily employed, a few corpuscles made their appearance in the blastema, a few were found in the inner layer of the bag, whilst the outer one contained a great many.

From these facts we are forced to the conclusion that the corpuscles migrated through the walls of the bags, just as they do to the interior of the cat-gut ligatures that are left in similar conditions.

This, however, is only a negative kind of proof, and for something positive I will ask the reader's attention to my recent study of the so-called adenoid tissue.

It is not necessary here for me to give the histology of the organs that contain this tissue, and to repeat that in the lymph glands it is arranged into lymph follicles, lymph cords, and interfollicular strings; in the alimentary canal

into follicles such as are contained by the tonsil, base of the tongue, pharynx, æsophagus, solitary glands, Peyer's patches, etc., etc.; in the spleen into the ensheathing coats of the arteries, and the so-called Malpighian corpuscles, etc., etc. But for our purpose, all that we need to know is that wherever this tissue may be there is a stream of fluid coming into it on one side, which, after working its way through the sponge-like mass, passes out on the other and eventually empties into the blood.

The two questions to which we will now address ourselves are: Whence comes and what is the function of the adenoid tissue?

All histologists agree that in the animal kingdom we find but four varieties of connective tissue and that they are the myxomatous, the fibrous, the cartilaginous, and the osseous. The myxomatous connective tissue is met with almost exclusively in the earliest stages of development of the embryonal connective tissue, and in transient fœtal organs, such as the umbilical cord and placenta. This tissue appears in two varieties: first, in the shape of a protoplasmic reticulum of greatly varying size, with nuclei at its points of intersection, the meshes of which hold the jelly-like mucoid basis substance (umbilical cord). In the centres of the meshes globular and apparently isolated bodies are seen. The other form consists of a delicate fibrous reticulum, having oblong nuclei at the points of intersection, the meshes being filled with single protoplasmic bodies (so-called decidua cells of the placenta), or with a mucoid basis substance with scanty bodies (derma and mucosa of the embryo in the earliest stages).

Recent researches have proved that this mucoid basis substance is not a structureless mass, but that it is pierced by a living reticulum, which is continuous with a smaller net-work which pervades all protoplasmic formations. As

the fibrous reticulum of myxomatous tissue is a protoplasmic formation, its fibres, too, contain a fine reticulum of living matter, which is also continuous with the fine reticulum of its neighbors. So the basis substance, in either its mucoid or fibrous variety, differs from protoplasm only by a chemically altered substance within the meshes. This substance in the protoplasm is a liquid, in the basis substance a semi-solid, though not strictly glue-yielding mass.

As has been known for a long time, comparatively low powers, when brought to bear on the adėnoid tissue, demonstrate the presence of a delicate fibrous reticulum, which at the points of intersection is generally slightly thickened and flattened so as to present a plate-like appearance.

These intersections are sometimes provided with nuclei, and the meshes of the net-work are always filled with lymph corpuscles. Although these corpuscles are so closely packed that they often flatten each other, still each one is generally separated from its neighbors by a narrow, light substance which is probably liquid.

Unless the lymph corpuscles be torn apart by mechanical injuries, such as cutting, washing, etc., etc., they are all connected with each other by extremely delicate, grayish spokes, which traverse the intermediate substance in all directions. A like connection always exists between the lymph corpuscles and the fibrous reticulum nearest to them. Most authors claim that this fibrous reticulum of the adenoid tissue is structureless, and exhibits nuclei only at its points of intersection.

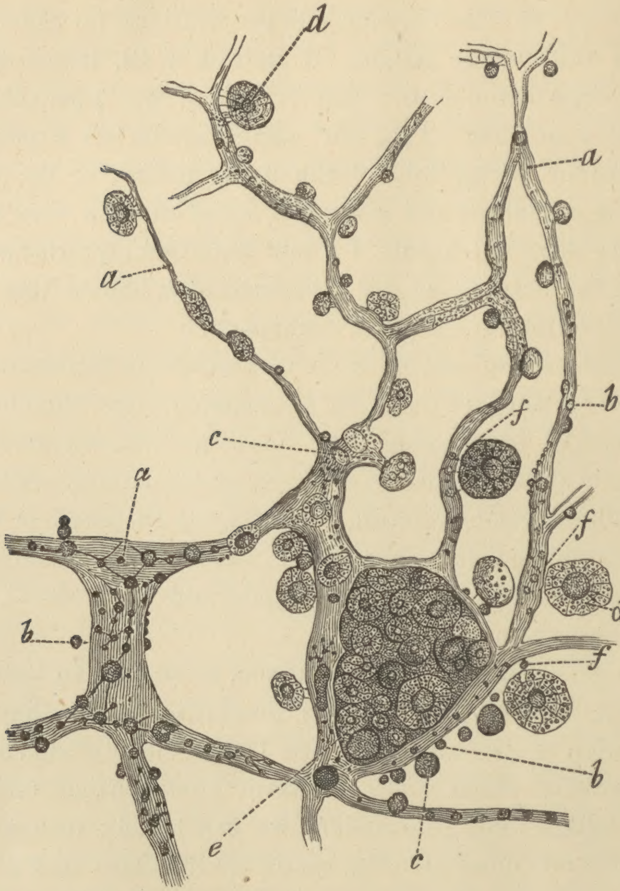
This assertion must be based on Canada balsam specimens, for it makes all minute details fade away. My own specimens, cut from fresh lymph glands, or such as had been preserved in a dilute solution of chromic acid, show a well-marked net-work in the fibrous reticulum both in the unstained and in the carmine specimens.

While we are on this subject of the preparation of specimens, let me say, once for all, that if we hope to see the minute structure of this tissue our sections must be cut from fresh or from chromic acid preparations, for alcohol or water destroys the details. If stained at all, it should be done with carmine, or what is better, the $\frac{1}{2}$-per-cent. of chloride of gold. This last named agent has a peculiar faculty for taking hold of the living matter of the most minute organisms and making it stand out in a very satisfactory manner. Lastly, I would state that glycerine seems to be the only mounting substance now known that will preserve tissues absolutely unchanged.

Reasoning by analogy it seems that we are forced to conclude that adenoid tissue is myxomatous, and, therefore, a remnant of fœtal tissue. We know that the myxomatous tissue is abundant in the embryo, and relatively scarce in the fully developed fœtus. In the adult the vitreous body was considered the only remnant of embryonal myxomatous tissue. To this, however, we should add the adenoid, and thus answer our first question.

To get a better idea of this tissue, let us turn to its most minute anatomy, and for the present we will confine our attention to its framework. As I have already said, in the framework, which looks perfectly homogeneous under a 500, with a 1,200 (immersion) we can readily recognize a delicate reticulum piercing nearly all its fibres and plates. In some places, even without the use of a staining reagent, this net-work is just as plain as in the corpuscles themselves, the only difference being that its meshes are a little wider than those in the globule. But the point to which I wish to draw particular attention is, that the granules, at its points of intersection, vary very much in size. Sometimes where they are seen along the edges of broad fibres, or in the centres of very fine ones, they give it a beaded appear-

ance. At others they are so small that they are just barely appreciable. This inequality in size is most probably due



Lymph ganglion of cat magnified 1200 diameters.
aaa. Myxomatous reticulum exhibiting in its interior a delicate reticulum of living matter.
bbb. Granules of living matter arising from the growth of the intersections of the contained reticulum.
ccc. Granules grown into vacuolized corpuscles, and intermediate stages of development.
ddd. Full-grown nucleated lymph corpuscles.
eee. Mesh of the myxomatous net-work filled with lymph corpuscles of all stages of development.
fff. Fine spoke-like threads connecting the corpuscles with the reticulum lying within the myxomatous framework.

to a growth that is constantly going on in these granules, and our finding different ones at different stages of it.

This process does not stop where the lump of living matter can be called a granule, but it keeps on until it has converted it into what is known as a corpuscle. This is accomplished by the smaller granule increasing until it has become so large that the fibre can no longer contain it without showing a slight bulging at the point where the granule lies. This is what gives the beaded appearance just referred to. But as the bead still grows it protrudes more and more from the free surface of the fibre, until it has the appearance of a small homogeneous yellowish corpuscle sticking to the side of the fibre. The corpuscle is not separated from the fibre in this immature state, but retains a connection in the shape of very delicate grayish spoke-like threads, that can be traced directly to the granules within the fibre. This connection is constant in all the different-sized corpuscles, except the very largest, and in all probability is the route through which the corpuscle draws its nourishment. We can see no differences in these growing corpuscles until they are about three-quarters the size of a red blood globule. Then, however, they seem to be divided into two classes. Whether there are two sets of fibres that produce the different corpuscles, or how else it is done, is more than I can say; but I am sure that at the stage I have indicated, one set become more highly refracting than the other, and take more and more of the characteristics of a red blood globule, which they eventually become. The others, however, follow the course that C. Heitzman has described (Sitzungsber. der Kais Akademie der Wissenschaften, 1873) as the course that the elementary homogeneous granule takes in its development into a higher grade of protoplasm. After they reach the size I have already spoken of, a cavity containing a small amount of liquid forms, then similar excavations show themselves, until only a framework of the living matter is left between the

vacuoles. There are communications established between these cavities, and the framework is transformed into a net-work with thickened points of intersection, which are the granules.

With this view of the development of protoplasm we are better able to understand the meaning of the vacuolized corpuscles that we so often meet with. But the different sizes of the corpuscles, the different numbers of their granules, and the varying conditions of their nuclei and reticula, speak for themselves. They are the different stages through which an original granule of the fine reticulum contained by the fibrous net-work is developed into a full-grown lymph corpuscle.

This is further substantiated by the fact that the connection, already described, between the granule that has just passed to the outside of the fibre and the reticulum within it, is kept up through all sizes and shapes of corpuscles, until the full-grown condition is reached. Then, however, this attachment is severed, and the globule passes away with the lymph stream in which it has been bathed so long. This is true of both sets of corpuscles, and can be shown as well in the young red, as in the white. Thus we add a new proof to the old idea that a red globule is nothing but a mass of protoplasm containing hæmoglobine within its meshes; but for the elaboration of this subject I refer my reader to the researches of L. Elsberg.

The organs that I have used in these investigations are the lymphatic ganglia of man, horse, and cat, the spleen of man and cat, as well as the tonsil and thymus gland of children. The characteristics of the adenoid tissue were found to be the same in all, the principal differences being in the proportion of red to white globules. In the tonsil and lymphatic ganglia, the red are very scanty, though they can be found in most fields; but in the spleen they are far

more frequent. In this organ, like the rest, the corpuscles are formed by the development of the granules of the network within the frame, and not by budding of the endothelial plates, as claimed by some. We are now ready to give the reason for the lymph of the efferent vessels containing so many more corpuscles than that of the afferent, as well as to say where the few red globules that are found in the lymph of the thoracic duct come from. The lymph stream, as it passes through each successive ganglion, carries along an increased number of the fully grown elements that have become detached from the parent fibre, and eventually empties them into this duct, through which they reach the blood.

In answering these questions, we are also giving the function of the adenoid tissue, which is to produce the corpuscular elements of the blood.

It has been known for a long time, that as age advances the adenoid tissue becomes more and more scarce, and that the mucous layers and other organs that were once so rich in it, at extreme old age present scarcely a trace. In reality, the thymus gland may be taken as the type of the whole class. For while their degeneration is by no means so rapid, still they all show a tendency to follow its example. This is most strikingly shown in the history of Peyer's patches, as has been brought out by the study of typhoid fever. From this we would conclude that a young animal is the best subject for the study of the adenoid tissue. This I can testify is the case, for as age advances the granules of the reticulum within the fibres become more scanty, and the reticulum itself is by no means so rich as in the early days of life. Thus we see that we live at the expense of our cytogenic tissue. Should it ever be conclusively proved that the white blood corpuscles share in the formation or repair of the structures of the body, we would then have

the complete chain of their history; for we are now sure that they represent only one stage of a development that is going on as long as life lasts, and I am not inclined to believe that this stage is the highest of the series. The conclusions that I have drawn from these studies are:

1st. We must have more and better proof before we can believe that a lymph corpuscle ever arises from a blastema.

2d. That both red and white blood corpuscles are developed from the granules of the reticulum of living matter within the fibres of all adenoid tissues.

3d. That in different organs there is a difference in the proportion of red to white globules that are produced.

4th. That the adenoid tissue is myxomatous, and, properly speaking, a remnant of fœtal life.

5th. That this tissue is stored-up material, from which the blood corpuscles are made throughout life.

6th. That it is highly probable that the exhaustion of this material plays an important part in senile atrophy, and the other torpid conditions of the aged.

Before closing this paper, I wish to acknowledge the kind assistance rendered me in its preparation by Dr. C. Heitzman of New York, in whose laboratory much of the microscopical work was done.

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